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Isolation of *Salmonella enterica* and Shiga-Toxigenic *Escherichia coli* O157 from Feces of Animals in Public Contact Areas of United States Zoological Parks▽

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The fecal prevalence of subclinical *Salmonella enterica* and Shiga-toxigenic *Escherichia coli* O157 among animals in human-animal contact exhibits at institutions in the United States accredited by the Association of Zoos and Aquariums was estimated to assess public health risk. The prevalence was less than 0.6% for both zoonotic pathogens among 997 animals sampled at 36 exhibits.

Animal exhibits are popular sources of entertainment and educational enrichment that provide opportunities for direct and sometimes close human-animal contact. Zoonotic enteric human disease outbreaks associated with animal exhibits have increased in the past decade in North America and Europe. These outbreaks are usually attributable to the protozoan *Cryptosporidium parvum* and to nontyphoid *Salmonella enterica* and especially to Shiga-toxigenic *Escherichia coli* (STEC) O157 bacterial infections (5, 18). At least 17 animal exhibit-associated (agricultural fair, petting zoo, or open farm) STEC O157 outbreaks have occurred in the United States since 1999, and these outbreaks have affected 1,317 people, caused 69 hemolytic-uremic syndrome cases, and killed two persons (5, 6, 8, 9, 11, 12, 13, 18, 21). Since 1990, there have been at least four animal exhibit *Salmonella enterica* outbreaks in the United States attributable to *Salmonella enterica* serovars Typhimurium and Enteritidis (5). The *Salmonella* serovar Enteritidis outbreak, which was associated with visiting a temporary exhibit of a Komodo dragon at a metropolitan zoo, affected 65 persons, mostly children (15). Exhibit-associated outbreaks, real or alleged, are costly to affected individuals and their families, affected venues and their insurance underwriters, and health service providers. They also represent a source of legal vulnerability to exhibitors.

The Association of Zoos and Aquariums (AZA) is a nonprofit organization of 211 (in 2005) zoos, aquariums, and wildlife centers in North America. The AZA inspects and accredits member institutions every 5 years for adequacy of facilities, veterinary care, safety, security, collection management, finances, research, and other factors (1, 2). AZA member zoos and aquariums attract ~142 million visitors annually, employ ~46,000 people, and maintain collections of ~800,000 animals (3). About half of AZA-accredited institutions have human-animal contact areas (e.g., children's zoos or similar types of interaction settings).

Human-animal contact exhibits are heterogenous. They vary in hygiene and sanitation practices, degree of supervision, extent of animal contact permitted, numbers and types of animals displayed, nature of exhibits (temporary, recurring, or permanent), facility design, and visitor management (10, 18). Human-animal contact areas at AZA-accredited institutions are probably more similar to each other than they are to non-AZA exhibits due to the standardization inherent in accreditation. The motivation for this study was to estimate the unknown fecal shedding prevalence of zoonotic *Salmonella enterica* and STEC O157 in animal populations in the relatively homogenous AZA human-animal contact settings. We hypothesized that the fecal shedding prevalence of both bacteria would be lower in animals in AZA human-animal contact areas than that in animals in production or agricultural fair environments. Commercial beef and dairy cattle and livestock displayed at agricultural fairs frequently have high (10% or greater) summer prevalence of both STEC O157 and *Salmonella* (4, 7, 14, 17; T. E. Wittum, J. E. Keen, G. Hansen, D. Mollenkopf, J. A. Funk, J. R. Dunn, J. L. Bono, and M. E. Fontenot, 84th Conf. Res. Workers Anim. Dis., abstr. 61, 2003).

AZA-accredited institutions in the United States with human-animal contact exhibits (typically children's zoos) were recruited to participate voluntarily and confidentially. Freshly (i.e., observed) voided or rectal feces acquired digitally (~50 g, if available) were collected from a census of all animals in contact exhibits by institution staff. Fecal culture for both *Salmonella* and STEC O157 was initiated within 24 to 36 h of collection. Samples were collected in the summers of 2003 and 2004, the peak visitor season at most participating zoos and the peak period of *Salmonella* and STEC O157 shedding in livestock in general (4).

For *Salmonella* isolation, feces samples (up to 10 g, as available) were preenriched in tetrathionate broth (TTB) containing 0.1% brilliant green solution for 24 h at 37°C, followed by selective enrichment of 100 μl of TTB in 10 ml Rappaport-Vassiliadis R10 broth (Difco Laboratories, Detroit, MI) for 24 h at 37°C. R10 broth was then dual streak plated (10 μl) onto EF-18 agar (Neogen Corp., Lansing, MI) and Rambach agar (CHROMagar, Paris, France) (20, 22). Plates were incubated for 24 h at 37°C. Up to five colonies per plate exhibiting

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typical *Salmonella* morphology on the selective agars were inoculated onto MacConkey agar and incubated for 24 h at 37°C. Lactose-negative colonies were biochemically phenotyped on Sensititre AP80 gram-negative identification plates (Trek Diagnostic Systems, Westlake, OH). Isolates confirmed as *Salmonella* by Sensititre were serogrouped with a limited set of anti-*Salmonella* monoclonal antibodies by enzyme immunoassays (16). One isolate per *Salmonella*-positive fecal specimen was *Salmonella* O and H antigen serotyped by the National Veterinary Services Laboratories (NVSL) (Ames, IA).

For STEC O157 isolation, feces samples (up to 10 g) were enriched in gram-negative (GN) broth containing vancomycin (8 mg/liter), cefixime (0.05 mg/liter), and cefsulodin (10 mg/liter) for 6 h at 37°C, followed by immunomagnetic separation with anti-*E. coli* O157 paramagnetic beads (Dynabeads; Invitrogen, Carlsbad, CA). Following immunomagnetic separation, bead aliquots (50 μl) were spread plated onto CHROMagar O157 agar containing 0.63 mg/liter potassium tellurite (TCA). Up to five suspect STEC O157 mauve-pink colonies per TCA plate were serotyped by enzyme immunoassays using anti-*E. coli* O157 and anti-*E. coli* H7 monoclonal antibodies followed by PCR assays for stx_1 and stx_2 (Shiga toxins), *eae* (intimin), $rfbE_{O157}$ (O157 O antigen), and $fliC_{H7}$ (H7 flagellum) genes (13, 17).

Thirty-six AZA-accredited institutions participated in the survey, 13 in 2003 and 23 in 2004. The institutions were geographically diverse, from 25 different states and all regions in the United States. Fecal specimens were collected from 997 animals (Table 1), averaging 28 samples per exhibit (range, 4 to 68). *Salmonella* was isolated from six animals at four zoos: three goats, one horse, one bovine animal (zebu calf), and one giraffe (Table 2). *Salmonella*-positive animals were immediately removed from exhibits, quarantined, and retested for *Salmonella* at 2-week intervals. All animals initially *Salmonella* fecal positive were culture negative on subsequent retesting except the zebu calf (Table 2), which remained fecal *Salmonella* positive, but with a different serotype, 2 weeks after initial testing. STEC O157 was isolated from a yak in isolation and quarantine facilities just prior to going on display (Table 2). The yak isolate was PCR positive for stx_1, stx_2, *eae*, $rfbE_{O157}$, and $fliC_{H7}$. This animal was permanently removed from the zoo. Presumably nonzoonotic *stx*-, *eae*-, *hly*- and $fliC_{H7}$-negative *E. coli* O157 isolates were obtained from three pigs, four sheep, and one horse at seven zoos (incidental finding).

STEC O157 and *S. enterica* isolate antimicrobial susceptibility was evaluated by Kirby-Bauer disk diffusion on Mueller-Hinton agar and commercial disks (Difco) (23). The antimicrobials and disk concentrations (in micrograms) are shown in Table 2, footnote *a*. Results were interpreted according to established criteria (19). There was no evidence of clinically relevant antibiotic resistance among the eight isolated enteric pathogens (Table 2).

These survey results indicate that summer fecal prevalence for both *Salmonella* (6 of 997 = 0.6%; 0.2 to 1.3 exact 95% confidence interval [95% CI]) and STEC O157 (1 of 997 = 0.1%; 0.0 to 0.6 95% CI) was low in human-animal contact settings at AZA-accredited institutions in both absolute and relative terms. *Salmonella* and STEC O157 were isolated from animals in 4 of 36 (11.1%; 3.1 to 26.1 95% CI) and 1 of 36 (2.8%; 0.1 to 14.5 95% CI) exhibits, respectively. The prevalence at AZA-accredited institutions was much lower than the prevalence typical for either pathogen in production or agricultural fair livestock.

TABLE 1. *Salmonella enterica* and Shiga-toxigenic *Escherichia coli* O157:H7 fecal isolation rates from a census of animals in human-animal contact areas at 36 AZA-accredited zoos in the United States in 2003 and 2004

Animal group and type	No. of samples collected	No. (%) of samples positive for:	
		Salmonella	STEC O157
Domestic livestock			
Goats (mostly pygmy breeds)	526	3 (0.6)	0
Sheep (many breeds)	192	0	0
Cattle (dairy and beef cattle and yaks)	49	1 (2.0)	1 (2.0)
Equids (horses, ponies, and donkeys)	59	1 (1.7)	0
Pigs	45	0	0
Subtotal	871	5 (0.6)	1 (0.1)
Exotic and wild hooved animals			
Vicunas and llamas	26	0	0
Cervids (deer and reindeer)	33	0	0
Camels	2	0	0
Giraffe and okapi	7	1 (14.3)	0
Antelopes	7	0	0
Subtotal	75	1 (1.3)	0
Small animals			
Rabbits	16	0	0
Rodents[a]	10	0	0
Pigeons	3	0	0
Parrots	3	0	0
Tortoises	5	0	0
Poultry	11	0	0
Subtotal	48	0	0
Carnivores			
Skunks	1	0	0
Servals	1	0	0
Ferrets	1	0	0
Subtotal	3	0	0
All animals	997	6 (0.6)	1 (0.1)

[a] Rats, porcupines, chinchillas, and guinea pigs.

The low prevalence of *Salmonella* and STEC O157 at AZA-accredited institutions could result from the standardized management and facility conditions, routine isolation and quarantine procedures, generally high hygiene levels, low animal stress due to exhibit permanency (e.g., lack of transport stress), and low rate of new animal introductions and animal mixing compared to temporary or reoccurring types of animal exhibits or production livestock settings. Most human-animal contact exhibits at AZA-accredited institutions are permanent venues, and AZA zoological parks frequently possess more human and

TABLE 2. Antibiotic resistance of *Salmonella enterica* serovars and STEC O157 isolated from animals in human-animal contact areas at AZA-accredited zoological parks against 11 antimicrobials[a] as determined by disk diffusion

Zoo	Animal	*Salmonella enterica* serotype (serogroup[b]) or *E. coli*	Antibiotic resistance profile
3	Goat	Serotype Infantis (C1)	Ampicillin
8	Yak	STEC O157:H7	Ampicillin
18	Horse	Serotype Javiana (D1)	Ampicillin, azithromycin
29	Goat	Serotype Newport (C2)	Ampicillin, tetracycline, azithromycin
29	Giraffe	Serotype Rubislaw[c]	Ampicillin, tetracycline
36	Goat	Serotype Rubislaw[c]	Ampicillin, tetracycline, streptomycin, azithromycin
36	Zebu calf	Serotype Javiana (D1)	Ampicillin, tetracycline
		Serotype Muenchen (C2)[d]	Ampicillin, tetracycline

[a] Antimicrobials and their levels in disks (in micrograms) were as follows: ampicillin, 10; chloramphenicol, 30; streptomycin, 10; sulfisoxazole, 300; tetracycline, 30; trimethoprim, 5; ceftiofur, 30; ciprofloxacin, 5; gentamicin, 10; neomycin, 30; and azithromycin, 15.

[b] Serogroup as determined with anti-*Salmonella* serogroup B, C1, C2-C3, D1, and E1 and anti-*E. coli* O157 and H7 monoclonal antibodies by enzyme immunoassay.

[c] Isolate nonreactive with anti-*Salmonella* serogroup B, C1, C2-C3, D1, and E1 monoclonal antibodies by enzyme immunoassay; serotype Rubislaw belongs to serogroup F.

[d] Isolated on repeat fecal sampling while the zebu calf was quarantined 2 weeks after initial isolation of serotype Javiana.

financial resources than other animal exhibitors. Furthermore, many AZA institutions are in urban and suburban locations, distant from farms with endemically infected livestock. This spatial buffer may insulate them from rural infection pressures. Although *Salmonella* and STEC O157 screening is not part of routine AZA veterinary protocols for new or resident animals, existing AZA isolation and quarantine policies may protect against introduction and transmission of these nontargeted zoonotic agents as well. Finally, animals in contact areas at AZA-accredited institutions may be relatively free from these enteric zoonotic bacteria only fortuitously. Because of this possibility and because these infections are usually clinically silent, zoological parks should consider implementing a specific preventive zoonotic microbial screening program on a routine basis.

Differences in the compositions of animals examined in the present zoo study versus production or agricultural fair livestock surveys may also have impacted the findings. Adult cattle and swine are frequent targets of zoonotic enteric pathogen prevalence surveys at farms and fairs (4, 7, 14, 17), while sheep and goats are comparatively rarely surveyed, reflecting their relative agroeconomic importance. In contrast, the present zoo survey was 56% goats, 20% sheep, 5% cattle, and 5% swine. Compared to commercial livestock, livestock at AZA-accredited institutions also tended to be younger and smaller (e.g., miniature breeds) and managed in smaller groups for visitor safety and appeal. Nevertheless, the prevalence of STEC O157 and *Salmonella* in the surveyed zoos was low, even in the cattle and swine animal subsets most similar to commercial livestock.

In conclusion, human-animal contact exhibits at AZA-accredited institutions appear to present a low enteric zoonotic bacterial risk to their visitors and employees at the current time. These survey findings indicate that it is possible to maintain livestock species with low zoonotic enteric bacterial infection levels. Understanding the basis for this low prevalence could benefit preharvest food safety efforts aimed at lowering enteric zoonotic bacterial occurrence in farm livestock destined for food, a largely intractable problem to date.

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standards of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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